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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER
2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)
Malnutrition and Immune Response.

5. TYPE OF REPORT & PERIOD COVERED
Interim rept.

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)
Beisel, William R. Beisel

8. CONTRACT OR GRANT NUMBER(s)

9. PERFORMING ORGANIZATION NAME AND ADDRESS
U.S. Army Medical Research Institute of Infectious Diseases
Fort Detrick, Frederick, Maryland 21701

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
3M762776A841
A841-00-001

11. CONTROLLING OFFICE NAME AND ADDRESS
U.S. Army Medical Research and Development Command, Office of The Surgeon General
Department of the Army, Washington, DC 20314

12. REPORT DATE
4 January 1978

13. NUMBER OF PAGES
40

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)
44 p.

15. SECURITY CLASS. (of this report)
UNCLASSIFIED

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)
Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES
This is an invited Chapter for: An International Review of Biochemistry volume entitled "Biochemistry of Nutrition," to be published by The Medical and Technical Publishing Company, Ltd., of England. Eds. A. Neuberger and Thomas H. Jukes

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Malnutrition	Cell-Mediated Immunity
Protein Malnutrition	Antibodies
Energy Malnutrition	Lymphocytes
Pyridoxine	Host Defenses
Pantothenic Acid	

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
There can be no doubt that malnutrition has an adverse impact on immunological functions and can serve to suppress cell-mediated, humoral, and secretory immune competence. While most clinical studies have been performed in patients with generalized forms of malnutrition, immunosuppression has been found to occur with deficiencies of essential single nutrients.

Rarely does even a severely malnourished child exhibit the degrees of impairment in any immune function comparable to those experienced in →

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

congenital, primary immunodeficiency states. Further, nutritional immunodeficiencies in human beings appear to respond well to dietary therapy, with improvement in some functions becoming evident within a few days of initiating a refeeding program.

No entirely satisfactory animal model has yet been developed to replicate human forms of malnutrition. Nevertheless, animal models have considerable potential usefulness for probing the mechanisms of nutritional immunosuppression at the molecular level.

MALNUTRITION AND IMMUNE RESPONSE

By

William R. Beisel, M.D.

Running Head: Malnutrition and Immune Response

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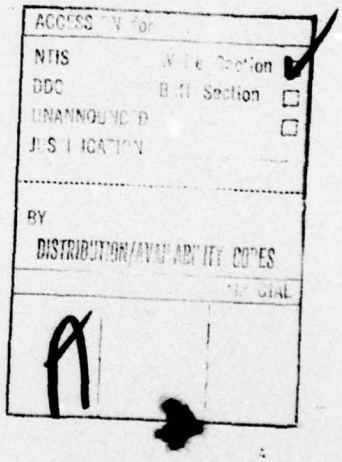

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1. INTRODUCTION

The adverse effects of malnutrition on host resistance against infection have been widely documented.[1] Although this relationship appears to be increasing in its clinical importance among adults who become malnourished as a secondary consequence of severe disease or trauma, the combination of malnutrition and infection is encountered most frequently in the infants and young children of impoverished societies. Not only do malnourished children have a higher frequency of diarrheal and respiratory infections, but the infections they develop tend to be more severe and prolonged.[2]

The mechanisms used by a normal host to resist infection seem to be impaired to some degree by severe nutritional deficits. Although malnutrition can reduce the effectiveness of nonspecific defensive mechanisms and lead to structural defects in anatomical barriers, such as epithelial and mucosal surfaces, the most important causes for an impairment of host resistance can be found within the areas of immunological function.

The effects of protein-energy malnutrition on immunological competence were not widely recognized until recently, despite the long-standing availability of published data describing the consistent presence of anatomical defects in lymphoid organs and tissues in malnourished children and laboratory animals.[3-5] However, in concert with recent technological and conceptual

advances in the field of immunology, systematic immunological studies have been carried out in pediatric malnutrition clinics in Africa,[6-12] Thailand,[13-17] India,[18-24] and the Americas.[22,25-28] Similar investigations have also begun in malnourished adult patients. These types of studies are already providing direct clinical benefits for patients with serious and chronic illness. Surgical teams, for example, are finding it of value to survey their patients with a variety of nutritional and immunological tests, and to use vigorous supportive nutritional therapy to improve or correct host defenses, including immunological functions.[29-31]

Thus, most of the information available for this review has come from clinical studies performed in man. The potential usefulness of experimentation in animals, on the other hand, has not as yet been fully utilized to determine mechanistic details concerning the interrelationships between malnutrition and specific immunological dysfunctions. As of the present time there are no completely satisfactory animal models available for studies of these interactions. Nevertheless, it is possible, through nutritional manipulations under controlled conditions, to influence both the function and numbers of T- and B-lymphocytes in laboratory animals and change the serological and cell-mediated responses to standardized antigens.[32] Interpretation of animal data and their applicability to man will have to take

into account the differences in immunological response which can be attributed to the type of nutritional deficit, its speed of onset and duration, as well as the differences caused by the species of animal used for a study.[32]

The complex interrelationships between malnutrition and immunocompetence were evaluated in detail during a 1976 Kroc Foundation Workshop, the proceedings of which were published as a monograph edited by R. M. Suskind.[33] Other recent reviews have also been stimulated by the importance of the problem.[4,34-38]

2. CHANGES IN LYMPHOID TISSUES

The widespread anatomical changes in lymphoid organs that accompany various generalized forms of malnutrition provide evidence of immunological cell dysfunction which is relatively easy to identify and quantitate.

Atrophy of the thymus gland in malnourished children was described as early as 1845 by Simon[5] who taught that the thymus was a very delicate barometer of malnutrition. Although thymus involution has consistently been viewed as a key indicator for the severity of malnutrition, the spleen, lymph nodes, tonsils, appendix, Peyer's patches and other lymphoid tissues may also become atropic. Clinical examinations of severely malnourished children revealed a prevalence of 36 to 65% in whom the tonsils could not be visualized or were only "trace" in size.[9] Such atrophic changes

can generally be reversed by refeeding, although the rate of recovery of individual thymolymphatic tissues is variable in different species.

During generalized malnutrition, thymic involution can advance to a point where virtually all lymphoid elements and Hassal's corpuscles are replaced by fibrous stroma. The anatomical changes in other lymphoid organs involve primarily the thymic-dependent areas. The depletion of T-lymphocytes in the paracortical areas depends to some degree upon the severity of malnutrition. Studies in guinea pigs show the paracortical lymphocytes to have slowed turnover and mitotic rates.[39] Reduction in the size and number of germinal centers is also noted. B-cells in perifollicular areas may be fewer but plasma cell numbers in medullary areas are usually maintained.

Variable reductions have been reported in the number of lymphocytes circulating in peripheral blood during malnutrition, with the greatest reduction occurring in spontaneously rosetting, thymus-dependent T-cells.[3,9,17,18,34,40-43]

The combination of anatomical findings and changes in peripheral blood lymphocyte counts is compatible with the view that malnutrition has a greater impact on the function of T-cells and cell-mediated immunity than it does on B-cell function and antibody-synthesizing mechanisms.

3. CELL-MEDIATED IMMUNITY

Generalized nutritional deficits appear to have a clearly adverse effect upon cell-mediated immunity (CMI). This arm of the immunological system includes the many immune phenomena that are dependent upon T-cell function for their induction and expression. Subpopulations of T-lymphocytes include killer, suppressor, and helper cells. The adverse effects of malnutrition on CMI have only been recognized during the past decade, but appear to affect lymphoid tissue anatomy, allograft rejection, delayed dermal hypersensitivity, T-cell numbers, and *in vitro* tests of lymphocyte function. Since the T-cell functions appear to be essential components for host defense against many viruses, fungi and facultative intracellular mycobacteria, it is not surprising that infections such as tuberculosis, measles, herpes simplex, vaccinia, and pneumocystis pneumonia all exhibit a more virulent course when they occur in malnourished children. Further, a variety of tests conducted *in vivo* or *in vitro* combine to show that, in human beings, T-lymphocyte function and CMI is compromised by malnutrition.[3,4,7-12,17,18,22,25,27-38] Similar findings have also been obtained in laboratory animals.[7,23,32,44-52]

Many of the tests used clinically for assessing T-cell functions or other parameters of immunological competence are altered by the presence (or recent occurrence) of an infection. Since infections

are commonly present in malnourished patients, published data are often difficult to interpret because no information is given concerning the presence or absence of infectious diseases in the patient population under study.

3.1 Circulating T-Lymphocyte Numbers

Actual counts of lymphocyte subpopulations consistently indicate that the number of T-cells, identified by their ability to form rosettes with sheep red-blood cells, is diminished in patients with marasmus or kwashiorkor.[3,8,9,17,28,36,37,41-43] A similar depression in numbers has also been noted during malnutrition in several animal species.[51] In the presence of normal or slightly depressed total lymphocyte counts in peripheral blood, the depression in T-lymphocyte numbers appears to be made up, primarily, by lymphocytes lacking T- or B-cell surface markers (null cells). The T-cell percentages fall to about one-fourth to one-half of their normal values in patients with severe protein-energy malnutrition.

3.2 Delayed Dermal Hypersensitivity

Delayed dermal hypersensitivity to various common antigens is one of the most widely used *in vivo* tests for evaluating CMI. A positive response requires prior sensitization to the antigen and is manifest by the delayed development of localized dermal infiltration by mononuclear cells and the accumulation of

protein-rich fluid. The magnitude of the response is best evaluated by measuring the diameter of palpable dermal thickening at 48 hours. Strongly positive responses may progress to the point of dermal necrosis.

The ability of a previously sensitized patient to develop a positive response is commonly lost during generalized malnutrition[3,6,9,11,12,24,27,29,42,49] if the patient is tested by an intradermal injection with one or more of the ubiquitous antigens such as tuberculin, Monilia, trichophyton, Candida, mumps, streptokinase-streptodornase (SK-SD), or phytohemagglutinin (PHA).

Neumann and her colleagues[9] found that the incidence of delayed dermal hypersensitivity to PHA was reduced to 44 to 45% in a group of severely malnourished Ghanaian children. This was in marked contrast to an almost 100% positive response in normal American children. The inability to manifest delayed dermal hypersensitivity may be partial, or it may be dose-dependent, requiring larger quantities of antigen to elicit a positive response. Dermal hypersensitivity responsiveness is generally restored progressively as nutritional deficits are corrected.

Attempts to utilize delayed dermal hypersensitivity responses as clinical indicators of CMI are influenced by a lack of knowledge concerning the prior immunological experience of the

patient (i.e., Does he have the prior sensitization required for a test to be positive?), by the dose of an antigen being used, and by the possible presence of a concomitant infection. In emphasis of this latter point, Schlesinger et at.[27] found that only 1 of 12 marasmic children had a positive tuberculin skin test despite the fact that all had received a BCG vaccination at birth. This finding contrasted to a positive dermal response in 46 of 52 healthy vaccinated control children, but the results in malnourished children did not differ significantly from a finding of only two positive skin tests among eight normally nourished children who had an extraneous acute infection at the time of testing.

A positive delayed dermal hypersensicivity reaction requires a functional response by each of three independent component portions of the basic response mechanism.[3] These include sensitization, recognition (elicitation or recall), and inflammation. All three components may be disrupted, singly or in combination, in the presence of malnutrition.[3]

Patients with severe protein-energy malnutrition may not become sensitized by an antigen to which they would normally respond. The sensitization component can be tested in vivo by administering an unusual or unfamiliar antigen (such as keyhole limpet hemocyanin) to a patient or experimental animal, and determining if dermal hypersensitivity will develop within a 2 to 3 week period. Using

such a test sequence, Smith et al.[11] found that 71% of a group of 17 severely malnourished children failed to become sensitized, whereas all control subjects developed positive tests. A deficient ability to become sensitized will generally respond rapidly to successful dietary therapy despite the initial severity of the malnutrition.[9]

Malnourished patients who were previously sensitized to an antigen may fail to respond to it because their immunological recognition mechanism is faulty. This type of deficit can generally be detected clinically by skin-testing patients with one or more of the ubiquitous antigens in a range of doses.

Even if both the sensitization and recognition limbs of a delayed dermal hypersensitivity response were intact, a positive reaction would not occur if the functional mechanisms for generating a localized area of inflammation were impaired. An inflammatory response is initiated in large measure because of the localized release from injured cells and "activated" lymphocytes of chemotactic factors and other chemical mediators. Children with severe protein-energy malnutrition may fail to develop a localized inflammatory response[3] on dermal testing with nonspecific irritant doses of chemicals such as 2,4-dinitrofluorobenzene (DNFB), or 2,4-dinitrocholorobenzene (DNCB). Monocytes of malnourished children infiltrate only sluggishly into areas of inflammation,[3]

and their release of (or response to) endogenous mediators of inflammation seems to be abnormal.

The possibility that delayed dermal hypersensitivity responsiveness can be transferred passively to a severely malnourished person remains an unsettled question. Brown and Katz[6] inoculated 12 African children suffering from either kwashiorkor or marasmus and 5 healthy controls with transfer factor extracted from the peripheral lymphocytes of a tuberculin-positive subject; all recipients developed a positive tuberculin skin test. This study has been criticized, however, on several grounds, including the ambiguous nature of transfer factor *per se*, a possible transient booster effect caused by closely repeated skin tests, and the fact that the malnourished children under study were receiving nutritional therapy during the course of the studies.[3] In a subsequent randomized double-blind clinical trial which compared transfer factor injections with saline in 32 Guatamalan children who were recovering from protein-energy malnutrition, Walker *et al.*[53] failed to find a demonstrable effect of transfer factor on the recovery of delayed dermal hypersensitivity responses.

3.3 Lymphocyte Transformation Studies

Lymphocyte blastogenic transformation studies, performed *in vitro*, are of value for measuring the responsiveness of T-lymphocytes to familiar specific antigens or certain nonspecific mitogens.

Normal lymphocytes respond *in vitro* to the presence of nonspecific mitogens, such as phytohemagglutinin, by undergoing blastogenic transformation. This response can be quantitated by measuring the increased cellular uptake of [^{3}H]thymidine in PHA-stimulated cultures in comparison to responses of identical control cultures not exposed to the mitogen. Cells from a healthy subject, previously sensitized to an antigen, will also respond *in vitro* to the presence of that antigen by blast transformation. T-cells will also respond in mixed leukocyte cultures to surface alloantigens on cells of another person or individual of the same species, or in animal studies, to xenogenic cells. All three types of T-cell mitogenic responses may be lost or impaired in patients or animals with severe generalized nutritional deficits.[31]

When tested by these *in vitro* methods, the T-lymphocytes from malnourished patients are generally found to exhibit depressed blastogenic responses to mitogens.[3,4,8-10,17,18,24,27,29,30,34,36,42] This lymphocytic impairment correlates well in individual patients with depressed dermal hypersensitivity reactions, and can be corrected by appropriate nutrient replacement therapy. Mixed leukocyte cultures can also be used to assess the blastogenic responsiveness of T-cells. Use of this recently introduced technique in patients with marasmus provided additional evidence for T-cell deficiency.[31]

While much animal data appears to confirm the depression in mitogenic responsiveness observed in lymphocytes from malnourished patients,[3,4,32,47,48] some dietary manipulations that produce deficiencies in single amino acids or protein[3,32] appear to inhibit B-cell function without inhibiting cellular immunity. In fact, a depression of serum blocking antibodies in mice with protein deficiency appeared to enhance the effectiveness of cytotoxic lymphocyte actions against mouse tumors.[32]

3.4 Transplantation Immunity

The ability of a host to reject allografted tissue is dependent upon CMI functions, primarily through the actions of killer T-lymphocytes. This CMI function may also be depressed in malnourished animals, in which the grafted allogeneic tissues remain viable for longer periods of time.[46,51]

In a series of investigations concerning killer T-lymphocyte functions, Good et al.[32] found that allograft survival and killer cell function could be manipulated through dietary changes toward either an enhanced or a depressed apparent level of killer cell function. A chronic deprivation of dietary protein caused mice to reject either allogeneic spleen cells or skin grafts at an accelerated rate. The accelerated response could be abrogated by prior thymectomy. In contrast, killer cell functions were reduced by a short-term protein restrictions followed by high

protein feedings, or by diets with a chronic limitation in their essential amino acid content.[32] These paradoxical findings suggested that the several types or durations of nutrient deficiencies may be having a different effect on the production of blocking antibodies or cytotoxic antibodies versus the function of cytotoxic cell-mediated immunity.[32]

3.5 Lymphokine and Interferon Production

A large variety of diverse nonantibody substances are released by lymphocytes into the surrounding body fluids or tissue culture media. These substances, collectively termed lymphokines, include macrophage migration inhibitory factor, mitogenic factor, and other biologically active products. The lymphokines serve an important function in activating macrophages and are also believed to play a role in modulating other body defense and immunological functions. However, little is known about the molecular structure or function of lymphokines. The production or release of interferon from lymphocytes and other cells may be suppressed in malnourished patients[27] or animals.[3,54]

3.6 Nutritional Deficiencies Associated with Impaired Cell-Mediated Immunity

Although most clinical studies reporting cell-mediated immunosuppression have focused upon patients with generalized forms of malnutrition, there is also evidence, derived especially

from animal studies, that single-nutrient deficiencies can diminish immunological responsiveness. Pyridoxine deficiency in experimental animals produces thymic and lymph node atrophy, prolonged skin graft rejection times, impairment of both sensitization and recognition mechanisms for mycobacterial antigens, and impairment of in vitro lymphocyte responses to test antigens.[44-46] Deficiencies of other B complex vitamins including thiamine or pantothenic acid produce less clearly defined changes. Axelrod[46] suggested that pyridoxine deficiency has its principal effect on nucleic acid synthesis while pantothenic acid deficiency could interfere with the release of immunoglobulins from lymphoid cells into the surrounding fluids. No consensus has been reached on the role of vitamins A or C on CMI competence.[3]

Since zinc plays a role in nucleic acid synthesis and is also an essential component of many metalloenzymes, it is not surprising that a deficiency of body zinc could lead to impaired immunological functions. Balb/C mice fed a zinc-deficient diet developed a selective exaggerated decrease in the weight of all lymphoid organs in comparison to those of other organs of the deficient mice as well as in comparison to organs of control mice pair-fed a zinc-sufficient diet.[55] The zinc-deficient mice showed a delayed response in the number of splenic plaque-forming cells after immunization with sheep red blood cells, an antigen which

requires T-cell helpers in addition to a B-cell response.[55]

The familial disease, acrodermatitis enteropathica, is now known to be due to an inherited disorder in zinc absorption from the intestine. The disease is manifested by chronic dermal lesions, diarrhea, alopecia and eye lesions, with frequent superimposed yeast and bacterial infections, all of which can be dramatically reversed by correcting the zinc deficiency.[56] Although defective cell-mediated and humoral immune functions may contribute to the disease picture, recent case studies suggest that the immunological defects are not due to the zinc deficiency, per se, but to the generalized malnutrition that typically accompanies severe forms of the disease.[56,57]

Considerable evidence obtained in man suggests that deficiencies of iron and/or folic acid produce an impairment of lymphocyte function.[7,15] While the individual roles for each nutrient and their interactions have yet to be clarified with respect to CMI functions, there is evidence that severe iron deficiency impairs the ability of lymphocytes to respond, in vitro, to mitogens or antigens or to produce macrophage migration-inhibitory factor. Folic acid deficiencies are also said to impair delayed dermal hypersensitivity reactions in in vitro lymphocyte transformation responses.[7,58] Further, studies in rats with experimental deficiencies of lipotropic factors (choline, methionine, B_{12},

and folic acid) showed impairments in cell-mediated functions as assessed by atrophy of lymphoid tissues, diminished response to sheep RBC antigens, and impaired [^{3}H]thymidine incorporation by PHA-stimulated thymic and splenic cells.[58] This effect of lipotropic factors suggests that methyl group metabolism is intimately involved in the maintenance of adequate CMI.

These data, in combination, make it seem likely that some portion of the immunological incompetence ascribed to generalized deficiencies of protein or energy could well be due to coexisting deficiencies of specific nutrients.

4. HUMORAL IMMUNITY

Humoral immunity is dependent upon the ability of lymphocytes in the presence of macrophages to recognize foreign antigens and then to initiate the production of specific antibodies against them. In the course of their normal circulation via the blood stream, T- and B-lymphocytes enter lymphoid tissues, remain for about one day, leave via the lymph, and recirculate. If the highly individualized cell surface receptors of an individual lymphocyte match up with an antigen, allowing it to impinge upon the exterior lymphocyte surface membrane, the cell begins to replicate, producing large numbers of clonal daughter cells, which in turn undergo differentiation and transformation into immunoregulatory T-cells and mature, immunoglobulin-secreting plasma cells.

Humoral immunity can be assessed by a variety of methods. Conventional serological tests such as the complement fixation, neutralization, and hemagglutination reactions are most widely used to detect the presence of specific antibody that can interact with specific antigens. A different kind of information about heterologous antibody classes is obtained through quantitation of the several types of immunoglobulins in serum, i.e., IgG, IgA, IgM, IgD, and IgE. The ability of the host to initiate a serological response can be evaluated by *in vivo* tests using specific antigens administered as vaccines or toxoids. B-lymphocytes can be identified by the immunoglobulins on their surface and counted. Using more sophisticated tissue culture methods (i.e., the Jerne plaque assay), the actively secreting plasma cells in tissue homogenates from some species can be identified and counted on the basis of the type of specific antibody they produce.

The impact of various forms of malnutrition upon humoral immunity has been difficult to ascertain with certainty.[59] Some studies appear to suggest that humoral immune functions are actually enhanced by malnutrition. The possible presence of coexisting infectious or parasitic diseases may account for some of the increase in immunoglobulin production. In any event, data compiled from all available human and animal studies allows for important differences in their possible interpretations.

4.1 Evidence for Normal (or Enhanced) Humoral Immunity

Total B-lymphocyte counts in peripheral blood generally remain near normal values during severe protein-energy malnutrition.[4,9,35,36,38] B-cell populations in lymphoid tissues show far less depletion than do the T-cell populations, while total plasma cell numbers seem to be maintained. Plasmacytoid cells have been observed in the peripheral blood of malnourished children.[27,28]

The total gamma globulin concentrations in plasma may remain normal or be increased;[14,59,60] rarely are they said to decline. IgM values tend to be increased in some newborn infants with foetal malnutrition[25] and they may also be high in older children although low or normal values have been reported.[14,59,60] The IgG values of malnourished infants are often increased,[14,59,60] and may approach adult levels by one or two years of age.[34] IgA values in plasma often increase, but they may remain in the normal range or decrease.[14,59,60] Relatively few studies have been reported and published on IgD or IgE values but these suggest that concentrations of both are increased.[14,59,60]

In addition to the increase in serum concentration of several classes of immunoglobulins during malnutrition, some data describe increases in the titer of antibodies with a specific function. Good and his collaborators[32] have described a series

of experiments in laboratory animals in which they could manipulate the type, magnitude, and duration of a dietary deficit in a manner that would increase the production rate and titer of blocking antibodies or cytotoxic antibodies. Chandra[19] has reported an increased incidence in malnourished children of antibodies against various foods. He suggested that these antibodies resulted as a secondary consequence of gut mucosa atrophy and impaired production of secretory IgA, which in combination permitted the passage of whole or partially degraded food-protein molecules into the blood stream.

These several types of data can be interpreted to imply that humoral immunity may be enhanced and that B-cell functions remain intact despite generalized protein-energy malnutrition, or at the very least, that humoral immunity is sustained more effectively than CMI. On the other hand, it may be argued that serum IgG values tend to be low in malnourished children kept free of infection.[18] Greater-than-normal IgG concentrations could result from an extraordinarily severe and continuing exposure of the child to the multiple antigens associated with heavy parasitic infestations, repeated bacterial and viral infections, and an unfavorable social environment.[2,25]

4.2 Evidence for Suppressed Humoral Immunity

An absent, delayed, or inadequate development of serological

titers have been reported in studies following inoculation of malnourished individuals with standardized doses of well-characterized antigens. A variety of vaccines may fail to generate the expected serological titer values when given to malnourished patients.[14,20,21,24,30,32,37,60,62] These findings include impaired antibody responses to influenza, typhoid, and live measles, polio, and smallpox vaccines. Comparable impairments in humoral response have been reported in malnourished animals immunized with antigens such as flagellin, tobacco mosaic virus, and allogeneic red blood cells.[32,46,47,50-52,60-62] McFarlane and Hamid[51] found a marked decrease in both the primary and secondary immunological responses of malnourished rats given initial and booster inoculations of sheep red blood cells. The impairment in splenic T-cell numbers was greater than the suppression of antibody producing cells after the primary inoculation while the reverse was true after the second inoculation. Other indicies of CMI suggested a greater degree of T-cell depression than exhibited by B-cell functions.

Other studies in mice[50] suggested that while all aspects of antibody responses to new antigens were depressed by protein-deficient diets, the capacity to produce IgG was more vulnerable than the capacity to produce IgM.

These types of studies have focused upon primary responses to a

"new" antigen.[62] Little is known about the responsiveness of malnourished persons to secondary or booster stimuli with familiar antigens, but some malnourished patients with nondetectable antibody titers against an antigen may show an anamnestic-type booster response if administered an antigen.[60] This suggests that "memory" had been present due to some prior experience with the antigen.

Studies of humoral responsiveness are also confounded by differences in the molecular composition of nonviable antigens, the route of their administration, their physical state (soluble vs. particulate), or the presence and effectiveness of adjuvants. Live vaccines are especially hard to evaluate,[62] since they introduce multiple antigenic combinations, undergo "processing" by a variety of host cells, and achieve an ultimate antigenic mass which depends primarily upon organism multiplication within the host rather than on the initial dose of inoculated organisms. Some malnourished patients have been found to generate an apparently normal serological response to one live attenuated viral vaccine while at the same time failing to show any response to another.[62]

4.3 Nutritional Deficiencies Associated with Impaired Humoral Immunity

Although some studies reveal normal humoral responses after an antigenic stimulus, depressed responses are common in both human

and animal studies where the malnutrition may be generalized. A similar depression of response has sometimes been associated with an induced deficiency in a single nutrient. Impaired humoral responsiveness has been demonstrated in animals during induced deficiencies of pyridoxine or pantothenic acid but not vitamins A or C.[44-46]

In a series of papers published in the early 1960's, Hodges and his coworkers[63-66] described impairments in the serological responses to several vaccines administered to adult volunteers who had been rendered deficient in one or more specific nutrients. Antibody responses to tetanus and typhoid vaccines were poor in a volunteer eating a protein-deficient diet.[63] Men receiving a pantothenic acid-deficient diet, with or without an added pantothenic acid antagonist, showed depressed responses to influenza and tetanus vaccines, but had normal responses to typhoid antigens and normal skin allograft rejection.[64] Only minimal impairments to tetanus and typhoid vaccines were seen during pyridoxine deficiency[65] but volunteers with combined pyridoxine-pantothenic acid deficiencies developed depressed serum IgG concentrations and responded poorly to tetanus and typhoid O antigens, minimally to typhoid H, and excellently to live polio vaccine.[66] The defective responses were all corrected by restoring the two missing vitamins.

5. SURFACE IMMUNITY

The secretory antibody system must be considered as separate from the serum antibodies. It provides antibodies for body surfaces such as the respiratory system, and gastrointestinal and genitourinary tracts, and in body secretions including saliva and milk. Secretory IgA is manufactured locally by plasma cells situated near the point of secretion. The secreted molecule consists of two IgA molecules linked by a J-chain (also produced by the plasma cell) and a secretory component which is synthesized by epithelial cells and attached to the combined 5000,000 MW molecule prior to its secretion into a glandular duct. IgA from the serum is not incorporated into the secretory IgA molecule. Small amounts of IgG and IgE may also be synthesized locally and secreted via exocrine glands. The localized application of an unfamiliar antigen onto a mucosal surface can stimulate the induction of secretory IgA without the initiation of a humoral antibody response.

Relatively few studies are available to document changes in surface immunity in malnutrition, but these indicate deficiencies in the system.[13,20,60,67-69] Nasopharyngeal, lacrimal and salivary gland secretions show a selective reduction in secretory IgA relative to their content of total protein and albumin.[13,20,60,69] The most severely malnourished of a group of Colombian children had depressed secretory IgA values in tears, in contrast to IgG

values which were high.[69] The responses of secretory IgA were poor in malnourished children immunized with live attenuated measles or polio vaccines; in addition serum antibodies failed to appear or were delayed and of low titer.[60] The concentration of secretory IgA in the nasal washings demonstrated a modest but significant depression in Thai children[13] who had either generalized protein-energy malnutrition or vitamin A deficiency. Low values persisted throughout the period of nutritional repletion. The deficient secretory IgA values could not be correlated with serum IgA values, which were high,[13,69] or with the presence or absence of upper respiratory infections.[13]

6. AGE-RELATED CHANGES

Although the majority of studies which describe the effects of malnutrition on the immune response have been performed in weanling infants, nutritional immunodeficiencies may develop at any age.

6.1 Foetal Malnutrition

Mata *et al.*[2] has reviewed the evidence emerging from underdeveloped societies that foetal malnutrition often begins during gestation as a direct consequence of deficient maternal diets during pregnancy, continuous exposure to unsanitary conditions, and recurrent clinical or subclinical maternal infections which sometimes involve the placenta and fetus as well. These effects can combine to cause premature births or intrauterine growth retardation. Infants

with a birth weight or less than 2,500 g are at special risk and suffer the highest neonatal mortality rates. Many of these small babies already exhibit an increase in their serum IgM concentrations at the time of birth. This finding may be attributed to antenatal antigenic stimulation by maternal or intrauterine foetal infections.

Chandra[21,34] found that low birth-weight newborn infants could respond normally to tetanus and typhoid immunizations but their antibody response to live poliomyelitis vaccine was impaired. Reduction in both B- and T-lymphocyte numbers may be evident at birth[11,40] and in vitro lymphocyte responses to PHA may be impaired. Undernourished or undersized newborn infants may also fail to become sensitized if tested with DNCB. Subsequently the rate of physiologic decrease in their serum IgG concentrations may be exaggerated between the ages of 3 to 5 months.[21,34]

Experimental evidence in rats suggests that the effects of maternal malnutrition may be manifested in later generations. Prolonged partial restriction of caloric intake in rats resulted in slowed growth, lymphopenia, involution of lymphoid tissues and an impaired response to primary immunization.[61] Both the first and second generation offspring from these starved mothers subsequently showed an impaired response to immunization with sheep red blood cells manifested by a reduced appearance of serum hemolysin titers and a smaller number of antibody-forming cells in the spleen.[61]

6.2 Childhood Malnutrition

The breast-fed periods of infancy are usually free of severe malnutrition or serious infection in the underdeveloped societies. Breast-fed infants initially tend to grow rapidly while remaining in the growth tracks for body weight, height, and head circumference predicted from their weight at birth. Until weaning, the nursing infant is protected by the nutrients in maternal milk, by the presence of viable phagocytic cells and immunoreactive lymphocytes,[70] by the passive immunity afforded by secretory IgA and other antibodies, and by other substances such as complement, interferon, lactoferrin, and bifidus factor.[68] Reddy et al.[71] found the concentration of immunoglobulins and lactoferrin to be highest in colostrum, while lysozyme values increased throughout the period of lactation. There appeared to be no differences in the milk from well-nourished or under-nourished Indian women.[71]

Severe malnutrition and the immunodeficiencies described in earlier sections of this review begin to appear in the postweaning years as a result of both dietary deficiencies and the nutrient-wasting effects of repeated infections which often combine to produce a vicious cycle and high rates of childhood mortality.[1]

6.3 Adult Malnutrition

Nutritional deficiencies which occur during later life can also lead to impaired immunological functions. The important studies

of Law et al.[29] in surgical patients with secondary malnutrition showed deficiencies in both humoral and cell-mediated immune functions. Failure to manifest delayed dermal hypersensitivity and impaired in vitro lymphocyte responses to phytohemagglutinin gave evidence for T-cell dysfunction. Although total serum immunoglobulin concentrations were in the normal range, the patients failed to produce an early serum IgM response after immunization with keyhole limpet hemocyanin, and thus showed evidence of B-cell dysfunction as well. These combined system impairments responded promptly to nutritional repletion.[29,31,47]

Most studies performed in man suggest that nutritional immunosuppression can be reversed by the appropriate repletion of existing deficiencies. Several studies in experimental animals, however, suggest that long-term effects of postweaning malnutrition may be observed, including some that paradoxically seem beneficial. Good et al.[32] found that a simple reduction in caloric intake could double the life-span of $(NZB \times NZW)_{F1}$ strain mice which regularly die as the result of antigen-antibody complex deposition within the renal glomerular tufts. Similarly, a normally long-lived (C57BL/6J) strain of mice studied by Gerbase-DeLima et al.[48] had their life-span prolonged even further by restricting their caloric intake after weaning. The mice that were fed a fully nutritious, normal diet, but only on alternate days, showed a generalized immunosuppression throughout the first half of

their life-span. This was manifested by impaired lymphocyte blastogenic responses to B- and T-cell mitogens, by poor serologic responses to sheep red blood cell antigens, and by delayed skin allograft rejection times. These deficiencies gradually corrected themselves late in life in the undernourished mice, while at the same time, the normally fed control mice developed an immunosuppressed state as they aged. The control mice died earlier than the undernourished ones.

7. INTEGRATION OF THE IMMUNE SYSTEMS

Much recent research has been aimed at identifying and quantitating individual mechanistic aspects of T-cell and B-cell functions. Major progress has also been made in defining the structure of immunoglobulins, their interaction with antigens, and the nature of cell surface receptors. As these important revelations emerge, they must be fitted individually into their appropriate niche in our overall understanding of the complex interrelationships that exist within the immune systems and their integration with other mechanisms of host defense.

7.1 Specific Immunity

Immunological defensive mechanisms are unique in their ability to recognize and respond to the specific molecular configurations in the structure of individual antigens. The process of generating and maintaining an immune response to a specific antigen is a

complex one, complete with innate modulating systems of checks and balances analogous to those which help regulate the hormonal and neurotransmission systems. The cellular processing of antigens and their trapping within lymph nodes involves the interaction of various phagocytes, macrophages, and lymphocytes and the differentiation, maturation, and proliferation of B-lymphocytes in the presence of helper and suppressor T-cells and their secretory products. Virtually nothing is known about the primary impact of malnutrition on these regulatory mechanisms when considered at the molecular or biochemical level.

7.2 Nonspecific Defense Mechanisms

The immune system functions of a malnourished subject are undoubtedly influenced by the occurrence of concomitant changes in nonspecific host defense mechanisms.[68,72]

Malnutrition-induced changes in neutrophil functions include delayed chemotaxis and defective microbicidal activities,[26,68,72] although neutrophil recognition and endocytosis functions remain normal. Electrolyte transport across leukocytic membranes is impaired in protein-energy malnutrition.[26] Resting activity of the hexosemonophosphate shunt may be increased in neutrophils while the burst of activity which accompanies phagocytosis may be diminished. The release of acid phosphatases from intracellular neutrophilic lysosomes may also be impaired.[73] The mononuclear

cells of malnourished children contain diminished activities of the enzymes phosphoglycerate kinase and pyruvate kinase.[74] A decrease in the migration of macrophages into areas of inflammation can also contribute to delays in antigen processing.[16]

Several groups have described a reduction in total hemolytic complement activity during generalized malnutrition.[3,13] This defect is a reflection of the diminished concentrations in plasma of all individual components of the complement system except C_4 in children with severe protein-energy malnutrition.[3]

Concentrations of other proteins with known antimicrobial functions may be depressed in the serum of patients with severe protein-energy malnutrition. These include depressions of serum transferrin, lactoferrin, and lysozyme, and an impaired release of interferon from cells.[27,54,68,72] On the other hand, the increased hepatic production of acute-phase reactant proteins such as orosomucoid, $alpha_1$-antitrypsin, haptoglobin, C-reactive protein, and ceruloplasmin which normally occurs in response to infection or an inflammatory stimulus will continue to occur in children or experimental animals despite the severity of coexisting generalized protein-energy malnutrition.[72] Some of these acute-phase reactants, such as C-reactive protein, are now being recognized as modulators of immune functions. Alpha-fetoprotein of hepatic origin may similarly be increased in malnourished infants.[23]

Other nonspecific factors which could influence the immune systems include a malnutrition-induced increase in plasma glucocorticoid values, an inhibition of febrile and inflammatory responses, an altered integrity of such mechanical barriers to infection as body secretions and epithelial surfaces, and finally an altered composition of microorganisms which constitute the normal body flora.[68,72]

Since endocrine changes are known to accompany malnutrition, it is also possible that some of the observed immunodeficiencies could result, in part, as a secondary consequence of hormonal actions. An increase in plasma concentrations of total and "free" cortisol known to occur during severe protein-energy malnutrition could exert antilymphocyte effects. In support of this concept, McFarlane[8] has reported an increase in the uptake of [^{3}H]corticosteroid by the spleen and thymus of underfed growth-retarded rats.

8. SUMMARY

There can be no doubt that malnutrition has an adverse impact on immunological functions and can serve to suppress cell-mediated, humoral, and secretory immune competence. While most clinical studies have been performed in patients with generalized forms of malnutrition, immunosuppression has been found to occur with deficiencies of essential single nutrients.

Rarely does even a severely malnourished child exhibit the degrees of impairment in any immune function comparable to those experienced in congenital, primary immunodeficiency states. Further, nutritional immunodeficiencies in human beings appear to respond well to dietary therapy, with improvement in some functions becoming evident within a few days of initiating a refeeding program.

No entirely satisfactory animal model has yet been developed to replicate human forms of malnutrition. Nevertheless, animal models have considerable potential usefulness for probing the mechanisms of nutritional immunosuppression at the molecular level.

9. REFERENCES

1. Beisel, W. R., Blackburn, G. L., Feigin, R. D., Keusch, G. T., Long, C. L. and Nichols, B. L. (1977). Amer. J. Clin. Nutr., 30, 1203, 1439
2. Mata, L. J., Kromal, R. A., Urrutia, J. J. and Garcia, B. (1977). Amer. J. Clin. Nutr. 30, 1215
3. Edelman, R. (1977). In R. M. Suskind (ed.). Malnutrition and the Immune Response, pp. 47-75. (New York: Raven Press)
4. Faulk, W. P., Mata, L. J. and Edsall, G. (1975). Trop. Dis. Bull., 72, 89
5. Simon, J. (1845). A Physiological Essay on the Thymus Gland. (London: Henry Renshaw)
6. Brown, R. E. and Katz, M. (1967). J. Pediatr., 70, 126
7. Gross, R. L. and Newberne, P. M. (1976). Adv. Exp. Med. Biol., 73(Pt. B), 179
8. McFarlane, H. (1977). In R. M. Suskind (ed.). Malnutrition and the Immune Response, pp. 127-133. (New York: Raven Press)
9. Neumann, C. G., Stiehm, E. R., Swenseid, M., Ferguson, A. C. and Lawlor, G. (1977). In R. M. Suskind (ed.). Malnutrition and the Immune Response, pp. 77-89. New York: Raven Press)

10. Schopfer, K. and Douglas, S. D. (1977). In R. M. Suskind (ed.). Malnutrition and the Immune Response, pp. 123-126. (New York: Raven Press)

11. Smith, N. J., Khadroui, S., Lopez, V. and Hamza, B. (1977). In R. M. Suskind (ed.). Malnutrition and the Immune Response, pp. 105-109. (New York: Raven Press)

12. Heyworth, B. (1977). Trans. R. Soc. Trop. Med. Hyg., 71, 251

13. Sirisinha, S., Suskind, R. M., Edelman, R., Asvapaka, C. and Olson, R. E. (1977). In R. M. Suskind (ed.). Malnutrition and the Immune Response, pp. 195-199. (New York: Raven Press)

14. Suskind, R. M., Sirisinha, S., Edelman, R., Vithayasai, V., Damrongsak, D., Charupatana, C. and Olson, R. E. (1977). In R. M. Suskind (ed.). Malnutrition and the Immune Response, pp. 185-190. (New York: Raven Press)

15. Suskind, R. M., Kulapongs, P., Vithayasai, V. and Olson, R. E. (1977). In R. M. Suskind (ed.). Malnutrition and the Immune Response, pp. 387-393. (New York: Raven Press)

16. Kulapongs, P., Edelman, R., Suskind, R. and Olson, R. E. (1977). Amer. J. Clin. Nutr., 30, 367

17. Kulapongs, P., Suskind, R. M., Vithayasai, V. and Olson, R. E. (1977). In R. M. Suskind (ed.). Malnutrition and the Immune Response, pp 99-104. (New York: Raven Press)

18. Chandra, R. K. (1972). J. Pediatr. 81, 1194

19. Chandra, R. K. (1975). Arch. Dis. Child., 50, 532

20. Chandra, R. K. (1975). Br. Med. J., 32, 583

21. Chandra, R. K. (1975). Amer. J. Dis. Child., 129, 450

22. Chandra, R. K. (1977). In R. M. Suskind (ed.). Malnutrition and the Immune Response, pp. 111-115. (New York: Raven Press)

23. Chandra, R. K. and Bhujwala, R. A. (1977). Int. Arch. Allergy Appl. Immunol., 53, 180

24. Kielmann, A. A. (1977). In R. M. Suskind (ed.). Malnutrition and the Immune Response, pp. 429-440. (New York: Raven Press)

25. Mata, L. J. and Faulk, W. P. (1974). Bol. Of. Sanit. Panam., 77, 410

26. Patrick, J. and Golden, M. (1977). Amer. J. Clin. Nutr., 30, 1478

27. Schlesinger, L., Ohlbaum, A., Grez, L. and Stekel, A. (1977). In R. M. Suskind (ed.). Malnutrition and the Immune Response, pp. 91-98. (New York: Raven Press)

28. Keusch, G. T., Urrutia, J. J., Guerrero, O., Casteneda, G. and Douglas, S. D. (1977). In R. M. Suskind (ed.). Malnutrition the Immune Response, pp. 117-123. (New York: Raven Press)

29. Law, D. K., Dudrick, S. J. and Abdou, N. I. (1973). Ann. Intern. Med., 79, 545

30. Carpentier, Y., Delespesse, G., Janne, P., Mendès da Costa, P. and Collet, H. (1976). Acta Chir. Scand., 466(Suppl.), 122

31. Kahan, B. E. (1978). In P. L. White (ed.). Metabolic Aspects of Critically Ill Patients, (Chicago: A. M. A. Publications) (In press)

32. Good, R. A., Jose, D., Cooper, W. C., Fernandes, G., Kramer, T. and Yunis, E. (1977). In R. M. Suskind (ed.). Malnutrition and the Immune Response, pp. 169-183. (New York: Raven Press)

33. Suskind, R. M. (ed.). (1977). Malnutrition and the Immune Response. (New York: Raven Press)

34. Chandra, R. K. and Newberne, P. M. (1977). Nutrition, Immunity, and Infection. Mechanisms of Interaction. (New York: Plenum Publishing Corp.) (In press)

35. Douglas, S. D. and Schopfer, K. (1976). Clin. Immunol. Immunopathol., 5, 1

36. Faulk, W. P., Paes, R. P. and Marigo, C. (1976). Proc. Nutr. Soc., 35, 253

37. McFarlane, H. (1976). Proc. Nutr. Soc., 35, 263

38. Katz, M. and Stiehm, E. R. (1977) Pediatrics, 59, 490

39. Bhuyan, U. N. and Ramalingaswami, V. (1974). Amer. J. Pathol., 75, 315

40. Moscatelli, P., Bricarelli, F. D., Piccinini, A., Tomatis, C. and Dufour, M. A. (1976). Helv. Paediatr. Acta, 31, 241

41. Chandra, R. K. (1977). Pediatrics, 59, 423

42. Rafii, M., Hashemi, S., Nahani, J. and Mohagheghpour, N. (1977). Clin. Immunol. Immunopathol., 8, 1

43. Gleason, Jr., W. A. and Roodman, S. T. (1977). J. Pediatr., 90, 1032

44. Axelrod, A. E., Hopper, S. and Long, D. A. (1961). J. Nutr., 74, 58

45. Axelrod, A. E., Trakatellis, A. C., Bloch, H. and Stinebring, W. R. (1963). J. Nutr., 79, 161

46. Axelrod, A. E. (1971). Amer. J. Clin. Nutr., 24, 265

47. Dionigi, R., Zonta, A., Dominioni, L., Gnes, F. and Ballabio, A. (1977). Ann. Surg., 185, 467

48. Gerbase-DeLima, M., Liu, R. K., Cheney, K. E. and Walford, R. L. (1975). Gerontologia, 21, 184

49. Ghavami, N. A. and Mohagheghpour, N. (1975). Nutr. Metab., 19, 158

50. Malavé, I. and Layrisse, M. (1976). Cell. Immunol., 21, 337

51. McFarlane, H. and Hamid J. (1973). Clin. Exp. Immunol., 13, 153

52. Rao, V. G. and Padma, M. C. (1975). Indian J. Exp. Biol., 13, 168

53. Walker, A. M., García, R., Pate, P., Mata, L. J. and David, J. R. (1975). Cell. Immunol., 15, 372

54. Younger, J. S. and Salvin, S. B. (1973). J. Immunol., 111, 1914

55. Frost, P., Chen, J. C., Rabbani, I., Smith, J. and Prasad, A. S. (1977). In G. J. Brewer and A. S. Prasad (eds.). Zinc Metabolism. Current Aspects in Health and Disease, pp. 143-150. New York: Alan R. Liss, Inc.)

56. Hambidge, K. M., Walravens, P. A. and Neldner, K. H. (1977). In G. J. Brewer and A. S. Prasad (eds.). Zinc Metabolism. Current Aspects in Health and Disease, pp. 329-340. (New York: Alan R. Liss, Inc.)

57. VanGool, J. D., Went, K. and Zegers, B. J. M. (1976). Lancet, 1, 1085

58. Newberne, P. M. (1977). In R. M. Suskind (ed.). Malnutrition and the Immune Response, pp. 375-386. (New York: Raven Press)

59. Neumann, C. G., Stiehm, E. R. and Swendseid, M. (1977). In R. M. Suskind (ed.). Malnutrition and the Immune Response, pp. 191-194. (New York: Raven Press)

60. Chandra, R. K. (1977). In R. M. Suskind (ed.). Malnutrition and the Immune Response, pp. 155-168. (New York: Raven Press)

61. Chandra, R. K. (1975). Science, 190, 289

62. Katz, M. (1977). In R. M. Suskind (ed.). Malnutrition and the Immune Response, pp. 421-427. (New York: Raven Press)

63. Hodges, R. E., Bean, W. B., Ohlson, M. A. and Bleiler, R. E. (1962). Amer. J. Clin. Nutr., 10, 500

64. Hodges, R. E., Bean. W. B., Ohlson, M. A. and Bleiler, R. E. (1962). Amer. J. Clin. Nutr., 11, 85

65. Hodges, R. E., Bean, W. B., Ohlson, M. A. and Bleiler, R. E. (1962). Amer. J. Clin. Nutr., 11, 180

66. Hodges, R. E., Bean, W. B., Ohlson, M. A. and Bleiler, R. E. (1962). Amer. J. Clin. Nutr., 11, 187

67. Michalek, S. M., Rahman, A. F. R. and McGhee, J. R. (1975). Proc. Soc. Exp. Biol. Med., 148, 1114

68. Neumann, C. G. (1977). In R. M. Suskind (ed.). Malnutrition and the Immune Response, pp. 355-374. (New York: Raven Press)

69. McMurray, D. N., Rey, H., Casazza, L. J. and Watson, R. R. (1977). Amer. J. Clin. Nutr., 30, 1944

70. Schlesinger, J. J. and Covelli, H. D. (1977). Lancet, 2, 529

71. Reddy, V., Bhaskaram, C., Raghuramulu, N. and Jagadeesan, V. (1977). Acta Paediatr. Scand., 66, 229

72. Beisel, W. R. (1977). In R. M. Suskind (ed.). Malnutrition the Immune Response, pp. 341-354. (New York: Raven Press)

73. Felsenfeld, O. and Gyr, K. (1977). Amer. J. Clin. Nutr., 30, 1393

74. Das, M., Stiehm, E. R., Borut, T. and Feig, S. A. (1977). Amer. J. Clin. Nutr., 30, 1949